Poster Session Group III – Green

TPS 69 – Novel immunological biomarkers and therapies

1479
Lipopolysaccharide induces neovascularization and immunosuppression, and may be considered as therapeutic target for anti tumor therapy

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Background: Previous studies showed that endotoxin – lipopolysaccharide (LPS) is both angiogenic and immunosuppressing, thus promoting metastatic growth (MG). However, the role of LPS as a therapeutic target is unclear. We hypothesized that anti-LPS therapy may decrease MG.
Method: Murine model including 3 groups (25 each) of adolescent mice was used. Metastatic process was modeled by i/v injection of 200 μl spontaneously metastasizing mammary adenocarcinoma cell culture suspension. Control group (CG) animals received 200 μl sterile saline intraperitoneal (i.p.), experimental group 1 (EG1) – 200 μl suspension of 10 μg LPS per mouse, experimental group 2 (EG2) – same plus 20 μg at 0.5 ml anti-LPS monoclonal antibodies. MG evaluated hystochemically within lung metastases.
Results: EG1 showed significantly higher ($P < 0.001$) MG compared with the control. MG was characterised by 61.2% higher mitotic index (MI) in the EG1 and 42.3% lower apoptotic index (AI). MI/AI ratio in the EG1 was 3.2 times higher ($P < 0.001$) than control. LPS injection resulted in reliably ($P = 0.002$) higher levels of serum VEGF than in control with strong positive correlation ($r = 0.971$) between circulating VEGF and LPS levels. Addition of anti-LPS monoclonal antibodies significantly decreased MG, MI and increased AI with respective change of MI/AI ratio. VEGF becomes insignificantly higher than in control whilst LPS concentration decreased reliably ($P = 0.014$).
Conclusion: Despite the well-established role of LPS as pro-inflammatory, pro-proliferator and pro-neovascularization factor, its role in carcinogenesis remains under evaluated. Our findings show that targeted anti-LPS therapy may impact tumor growth due to prevention of neovascularization and inflammation as well as inducing apoptosis.

1480
Clinical and immunologic efficacy of dendritic cell based immunotherapy in stage II breast cancer

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Background: Clinical and immunologic efficacy of dendritic cell (DC) based adjuvant immunotherapy was assessed in patients with Ki-67, p53 and HLA-A2 positive stage II breast cancer.
Methods: The protocol of clinical trials was approved by the Ministry of Health of the Republic of Belarus. There were 22 patients included in the trials who were treated with DC. DC were obtained from peripheral blood monocytes, primed with four p53 peptides and given by subcutaneous injection five times. Standard clinical examination of patients was done to exclude metastases. T-reg cells and antigen-specific T-cell (ASC) counts were assessed before and 6–30 months after the therapy.
Results: Safety and excellent tolerability of DC treatment was shown. In the current investigation there was an increase of ASC in 81.8 ± 8.2% of patients after the course of immunotherapy (before: 0.29 (0.07–0.5)%; after: 1.14 (0.7–1.67), $P = 0.0001$). The number of T-regs were decreased with treatment in 77.3 ± 8.9% of patients (before: 4.18 (2.67–5.99); after: 2.28 (1.85–2.97), $P = 0.0002$). After 3 years of DC-based therapy relapse-free survival was 95.4 ± 0.3% in patients treated with DC and only 75.4 + 1.5% in patients from a retrospective control group, suggesting DC efficacy in preventing metastatic breast cancer.
Conclusion: Clinical efficacy of DC based treatment of stage II breast cancer patients was shown. DC elicit the activation of anti-tumor immune response in the patients treated with dendritic cell immunotherapy.

1481
Extracellular vesicles modulate host-microbe responses by ligand-dependent inhibition of TLR2 activity

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Background: Lactic-acid-bacteria (LABs), including Bifidobacterium and Lactobacillus genera, have been proven beneficial in the maintenance of intestinal homeostasis. Ligation of Toll-like receptors (TLRs) expressed by resident dendritic cells (DC) to cell wall components expressed by LABs contributes to this mechanism of action. Extracellular vesicles (EV), important in cellular communication, originate from a broad range of cell types (including DCs) and can be found in virtually any body fluid. The reported presence of pattern-recognition receptors (including TLRs) on EVs, triggered the hypothesis that EVs can intervene with TLR activity.
Method: Heat-inactivated serum-derived EVs were collected using ExoQuick®. Intact human serum (HS), depleted serum (HS-D) and vesicle-containing pellets, reconstituted to the original volume with medium, (HS-EV) were collected. Monocyte-derived dendritic cells (moDC), THP-1 or HEK cells stably transfected with TLR2/TLR6, expressing an NFkB reporter construct were seeded in the presence of HS, HS-D or HS-EVs and stimulated with bacteria, TNFα or specific TLR2 ligands. After 16H NFkB activity (HEK-transfectants, THP-1) or cytokine release (moDC) was measured.
Results: *Bifidobacterium*, in contrast to *Lactobacillus* strains, induced TLR2 activity which was inhibited by HS or HS-EVs. EVs depletion rescued TLR2 activity. TLR2-heterodimer specific ligands showed that HS-EVs inhibition was TLR2/6 specific. Incubation of bacteria in the presence of HS and HS-EV, in contrast to medium or EV depleted serum, resulted in bacterial aggregation. Both Bifidobacteria and Lactobacilli induced dendritic cell IL-6 and TNFα release, which was either enhanced (Bifidobacteria) or reduced (Lactobacilli) upon EV depletion.